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STUDIES ON THE METABOLISM OF QUATERNARY PROTOBERBERINE ALKALOIDS IN CELL CULTURES OF *CORYDALIS PALLIDA* VAR. *TENUIS* AND *CORYDALIS INCISA*

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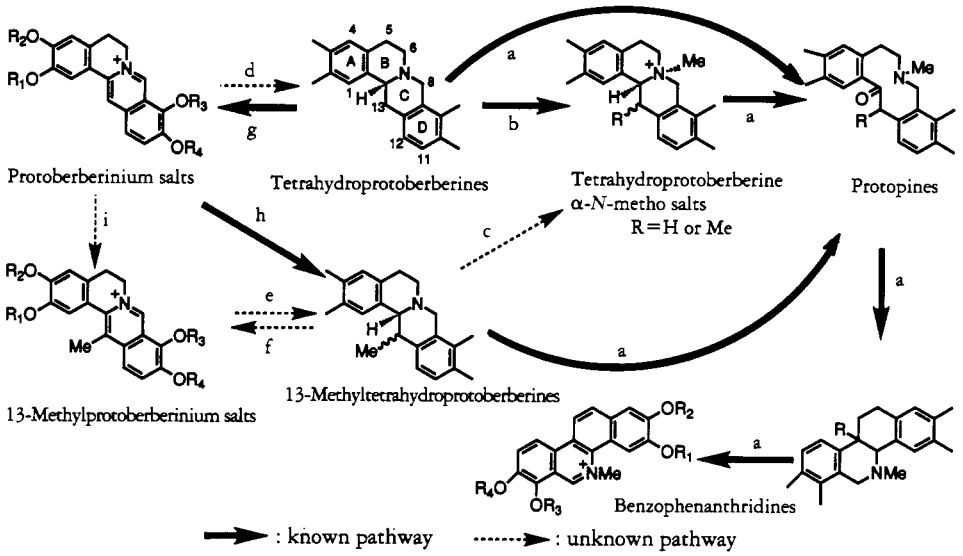
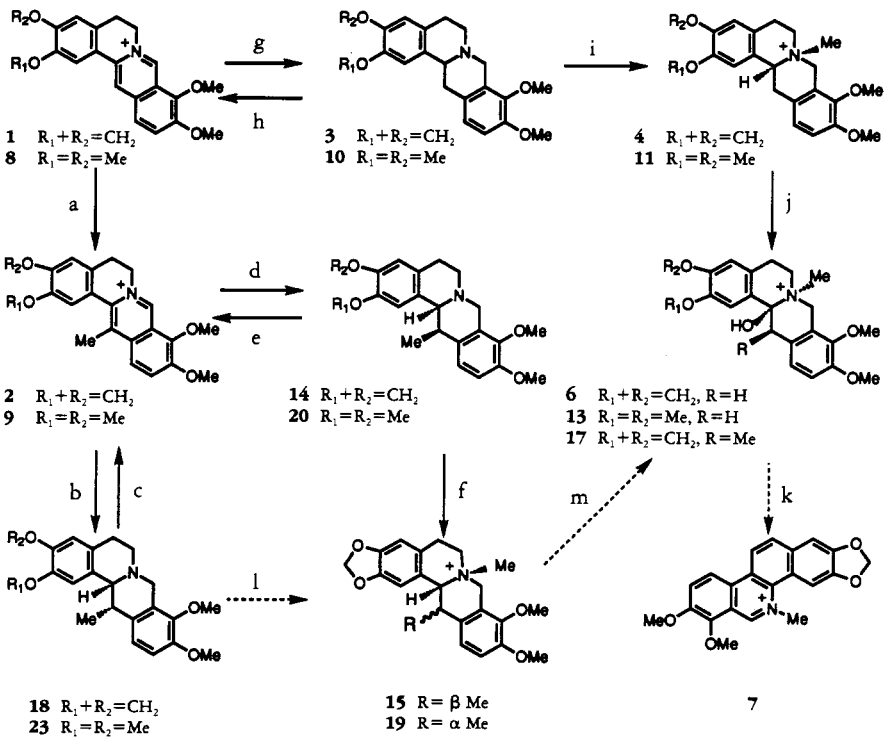
ABSTRACT.—An lc-apcims procedure was applied towards the identification of metabolites of quaternary protoberberine alkaloids in cultured cells of *Corydalis* species. Interconversions of tetrahydroprotoberberines [**3** and **10**] and protoberberinium salts [**1** and **8**] and those of *cis*- and *trans*-13-methyltetrahydroprotoberberines [**14**, **20**, **18**, **23**] and 13-methylprotoberberinium salts [**2** and **9**] were demonstrated. The oxidation-reduction process of the C ring of protoberberines was confirmed. The corresponding α-*N*-metho salts [**4**, **11** or **15**, and **19**] having the B/C-*cis* ring junction were produced from tetrahydroprotoberberines [**3**, **10**] or *cis*- and *trans*-13-methyltetrahydroprotoberberines [**14** and **18**]. Methylation at C-13 of the protoberberinium salts **1** and **8** afforded the 13-methylprotoberberinium salts **2** and **9**.

It has been demonstrated that tetrahydroprotoberberines, 13-methyltetrahydroprotoberberines, and their α-*N*-metho salts having the *cis*-B/C fused system, but not the corresponding *trans*-fused β-*N*-metho salts, are biotransformed via the corresponding protopines into benzophenanthridines in *Corydalis* species (route a in Scheme 1) (1). It is somewhat surprising, however, that α-*N*-metho salts have not been isolated from *Corydalis* spp. plants in general, except for *C. cava* (2). This may be attributed to the fact that α-*N*-metho salts are bioconverted rapidly into protopines. Protopines, but not α-*N*-metho salts, have been isolated as metabolites in incorporation experiments of tetrahydroprotoberberines using *C. incisa* (3). The formation of the α-*N*-metho salts of tetrahydroprotoberberines from the corresponding parent bases has been accomplished by Zenk and his coworkers using (*S*)-tetrahydroprotoberberine-*cis*-*N*-methyltransferase partially purified from an *Eschcholtzia californica* (Papaveraceae) cell suspension culture (4) (route b in Scheme 1). This is only one example of an α-*N*-metho salt isolated as a metabolite. The biosynthetic conversion of the 13-methyltetrahydroprotoberberines into α-*N*-metho salts was not demonstrated (suggested route c in Scheme 1). The redox interconversions between tetrahydroprotoberberines or 13-methyl–tetrahydroprotoberberines on the one hand, and protoberberinium salts or 13-methylprotoberberinium salts on the other, have not yet been fully investigated (suggested routes d–f in Scheme 1). The bioconversion of tetrahydroprotoberberines into protoberberinium salts has been demonstrated again by Zenk and his coworkers using (*S*)-tetrahydroprotoberberine oxidase which had been purified from *Berberis wilsoniae* (Berberidaceae) cell cultures (route g in Scheme 1) (5). However, demonstration of the reverse process (suggested route d in Scheme 1), namely, reduction, still remains to be accomplished. The *C*-methylation at C-13 was observed by Holland *et al.* (6) (route h in Scheme 1) in a study of the biosynthesis of corydaline [**20**] (Scheme 2) in *C. solida*. [9-$O^{14}CH_3$]-Palmatine [**8**], but not [9-$O^{14}CH_3$]-tetrahydropalmatine [**10**], was incorporated into corydaline [**20**]. Methylation at the C-13 site of protoberberinium salts might occur to furnish 13-methylprotoberberinium salts (suggested route i in Scheme 1). We undertook our present study in the belief that studies on the metabolism of these quaternary protoberberine alkaloids are essential for an understanding of the metabolic map of the protoberberine alkaloids, which are used medicinally as intestinal antiseptics (antibacterials) and stomachics.

Lc-apcims (liquid chromatography-atmospheric pressure chemical-ionization mass



SCHEME 1. Known and suggested routes for the formation of the quaternary protoberberine alkaloids in protoberberine biosynthesis.



SCHEME 2. Metabolic conversion in *Corydalis pallida* var. *tenuis* [routes l and m were not demonstrated; routes b, f, j, and k were demonstrated indirectly] and *C. incisa* [route k was not proved; routes a (**1**→**2**, b (**9**→**23**), i (**10**→**11**), j, and m were proved indirectly].

spectrometry) techniques were therefore applied to our metabolic studies of the quaternary alkaloids, which are highly polar and difficult to isolate.

RESULTS AND DISCUSSION

Protoberberinium salts, tetrahydroprotoberberines, their α- and β-*N*-metho salts, protopines, and benzophenanthridines were expected as the observed metabolites from the biogenetic pathways as presently understood. Prior to feeding experiments, authentic samples of the expected metabolites were divided into five groups [I–V] and lc-apcims was undertaken by SIM [selected ion monitoring] and TIM [total ion monitoring]. Group I consisted of the alkaloids [berberine [**1**], 13-methylberberine [**2**], tetrahydroberberine [**3**], its α- and β-*N*-metho salts [**4** and **5**], allocryptopine [**6**], and chelerythrine [**7**]] expected to be obtained from **1** or **3** by incorporating a methylenedioxy group on ring A. Indeed, in our hands, lc-apcims of group I showed a mass chromatogram having seven peaks (Figure 1, Table 1). Each alkaloid was identified by a quasimolecular ion $[M+H]^+$ or a cluster ion $[M+CF_3]^+$, or else a molecular ion $[M]^+$, and in each case by a characteristic retention time [R_t] (Table 1). Group II comprised expected products, namely, palmatine [**8**], 13-methylpalmatine [**9**], tetrahydropalmatine [**10**], its α- and β-*N*-metho salts [**11** and **12**] (Chart 1), and muramine [**13**], which can be derived from **8** or **10** and possess two methoxy groups in ring A. Group III consisted of the alkaloids **2**, thalictricavine [**14**], its α- and β-*N*-metho salts [**15** and **16**] (Chart 1), and 13-methyl-[*N*-$^{13}CH_3$]allocryptopine [**17**], which possess a methylenedioxy group on ring A and a methyl group at C-13, and which may be derived from either **2** or **14** (*cis* configurations of the protons at C-13 and C-13a). Group IV comprised the expected

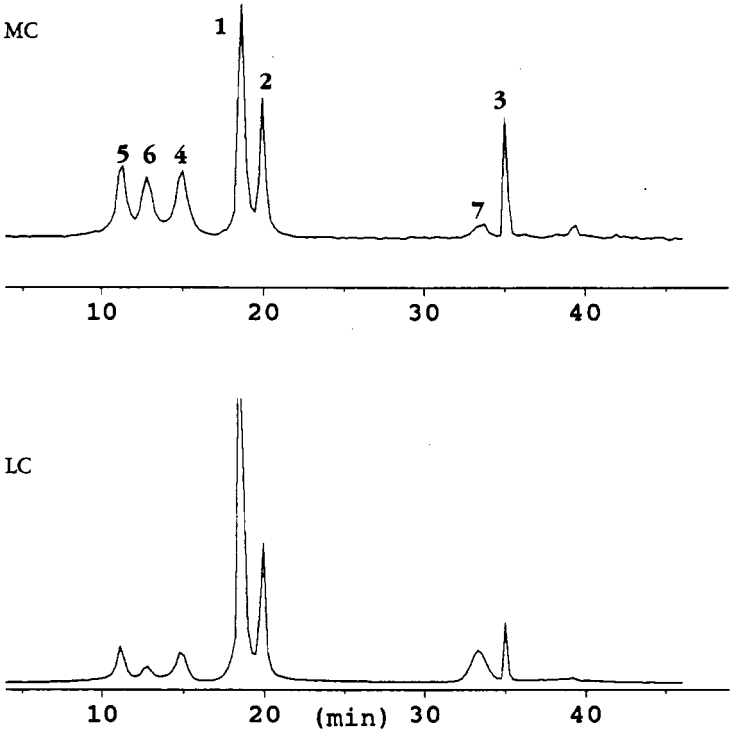


FIGURE 1. Mass chromatogram [TIC method, nebulizer temperature: 340°] and liquid chromatogram of Group I.

TABLE 1. Retention Times and Observed Ions by Lc-apcims [SIM Method] of Protoberberine and Related Alkaloids (Groups I–V).

	Observed ions (*m/z*)			
	R_t(min)	$[M]^+$	$[M+H]^+$	$[M+CF_3]^+$
Group I				
1	18.7			406
2	19.9			420
3	35.0		340	
4	15.0	354		
5	11.3	354		
6	12.8		370	
7	33.5	348		
Group II				
8	18.9			422
9	19.6			436
10	28.0		356	
11	13.0	370		
12	9.5	370		
13	11.0		386	
Group III				
2	20.0[a]			420
14	40.0		354	
15	18.1	368		
16	16.6	368		
[*N*-$^{13}CH_3$]-**17**	19.5[a]		385	
Group IV				
1	18.7[b]			406
2	20.0[c]			420
[*N*-$^{13}CH_3$]-**17**	19.7[c]	385		
18	32.7		354	
19	18.7[b]	368		
Group V				
9	19.7			436
20	33.7		370	
21	17.1	384		
22	15.3	384		
23	26.3		370	
[*N*-$^{13}CH_3$]-**24**	18.1	385		
[*N*-$^{13}CH_3$]-**25**	18.9		401	

[a,b,c]These peaks overlapped.

metabolites **1**, **2**, **17**, mesothalictricavine [**18**], and its α-*N*-metho salt [**19**], which have a methylenedioxy unit in ring A and a methyl group at C-13 and could be formed from **2** or, alternatively, **18** (*trans* configurations of the protons at C-13 and C-13a). Group V involved the alkaloids **9**, corydaline **20**, its α- and β-*N*-metho salts [**21** and **22**] (Chart 1), mesocorydaline [**23**], [*N*-$^{13}CH_3$]mesocorydaline α-*N*-metho salt [**24**] (Chart 1), and 13-methyl-[*N*-$^{13}CH_3$]muramine [**25**], which include two methoxy groups on ring A and a methyl group at C-13, and which could be formed from **9**, **20**, or **23**. Each alkaloid in groups I–V was identified by an observed mass spectral ion and by its R_t value (Table 1).

It should be noted that tertiary alkaloids such as the tetrahydroberberines and protopines showed quasimolecular ions $[M+H]^+$, quaternary alkaloids such as the protoberberinium salts cluster ions $[M+CF_3]^+$, and quaternary alkaloids such as the

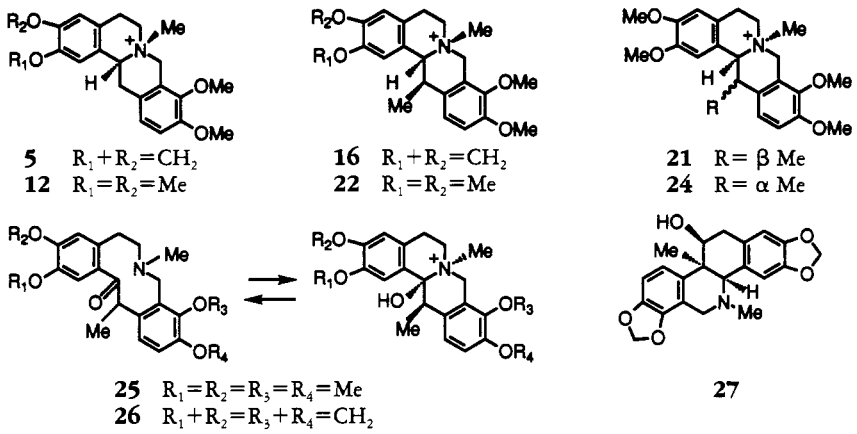


CHART 1

benzophenanthridines and the α- and β-*N*-metho salts of tetrahydroprotoberberines molecular ions $[M]^+$, under the lc-apcims conditions utilized (Table 1).

Feeding experiments with unlabeled protoberberines (Table 2) were carried out following confirmation that the expected metabolites were not present in cultured cells of *Corydalis pallida* (Pers.) var. *tenuis* (Papaveraceae). Callus tissues were incubated on an agar medium or in a liquid medium containing a substrate at 25° for an appropriate period of time (Table 2). A liquid medium was employed in feeding experiments using thalictricavine [**14**] and the related mesothalictricavine [**18**], corydaline [**20**], and

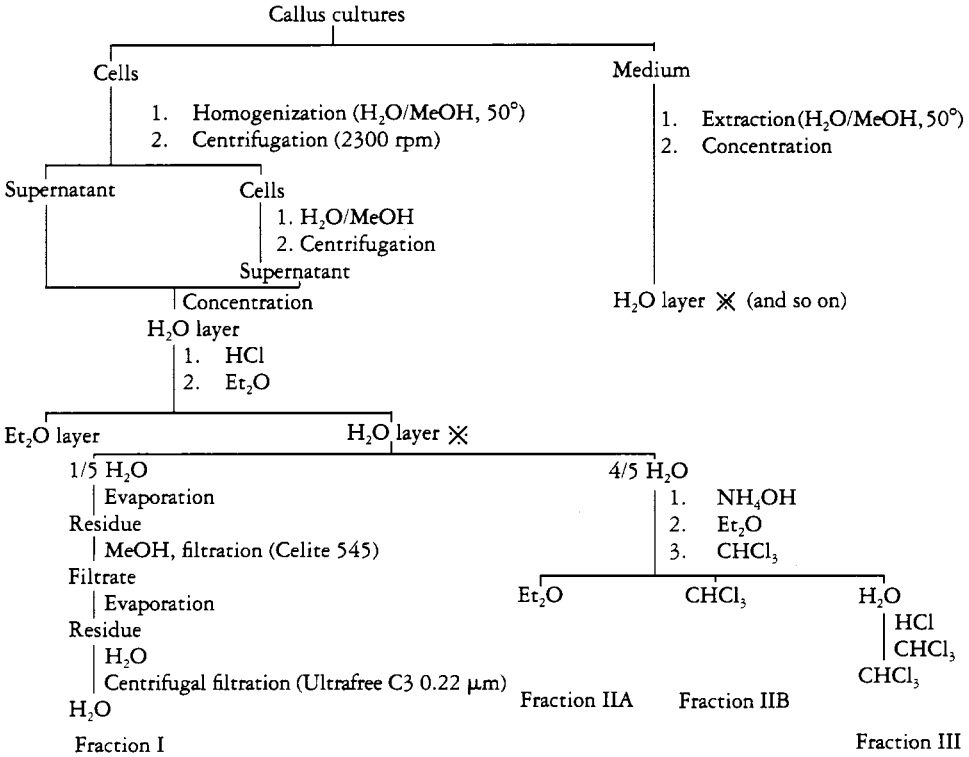


CHART 2

TABLE 2. Administration of Protoberberine Alkaloids to Cell Cultures of *Corydalis pallida* var. *tenuis*.

Experiment	Wt of dry cells (g)	Medium (ml)	Substrates (mg)		Incubation time (days)	Metabolite(s) detected [observed ions (*m/z*)]
1	4.62	800	**1**	100	24	**2** [420], **3** [340][a], **4** [354], **6** [370], **14** [354]
2	2.15	400	(±)-**3**	50	23	**1** [406][a], **4** [354][a], **6** [370][a], **6**-d_3 [373][a], **7** [348][a]
			L-[Me-d_3]-Met[b]	25		
3	4.37	800	**8**	100	20	**9** [436], **10** [356][a], **20** [370]
4	2.55	400	(±)-**10**	50	23	**8** [422][a], **9** [436], **11** [370][a], **13** [386][a] **13**-d_3 [389][a]
			L-[Me-d_3]-Met[b]	25		
5	2.59	400	**2**	50	21	**14** [354], **15** [368]
6	1.65	400	(±)-**14**	55	10	**2** [420], **18** [354]
7	1.67	400	(±)-**18**	55	10	**2** [420], **14** [354]
8	2.41	400	**9**	50	21	**20** [370]
9	3.78	400	(±)-**20**	55	10	**9** [436], **23** [370]
			L-[Me-d_3]-Met[b]	25		
10	3.00	400	(±)-**23**	55	10	**9** [436], **20** [370]
			L-[Me-d_3]-Met[b]	25		

[a]Metabolite has been also identified by lc-apcims (scan method) (7).
[b]L-[Me-d_3]-Methionine.

mesocorydaline [**23**], in order to preclude precipitation of the poorly soluble thalictricavine within an agar medium. Following incubation, the medium and cells were extracted according to the procedure shown in Chart 2. Fractions from the H_2O/MeOH extract [I], after removal of the neutral and acidic substances and pretreatment with a centrifugal filter unit [Millipore, Ultrafree C3], and alkaloid fractions [IIA, IIB, III] soluble in organic solvents, were subjected to lc-apcims [SIM method]. The metabolites identified in each fraction of experiments 1–10 are summarized in Table 2. Some metabolites from Experiments 1–4 had already been identified by ms analysis in the scan mode (7).

13-Methylberberine [**2**], tetrahydroberberine [**3**], its α-*N*-metho salt [**4**], allocryptopine [**6**], and thalictricavine [**14**] were identified in Experiment 1, in which berberine [**1**] was fed (Figure 2). Deuterated allocryptopine [**6**-d_3] and chelerythrine [**7**] as well as **1**, **4**, and **6** were detected in Experiment 2, in which tetrahydroberberine [**3**] was administered (Figure 2). The metabolic conversions **14**, **2**←**1**↔**3**→**4**, **6**, **7** were thus demonstrated in cultured cells of *C. pallida* var. *tenuis*. Berberine [**1**] was reduced to dihydroberberine which was methylated at C-13 to give 13-methyldihydroberberine. This was oxidized or reduced to afford **2** or **14**, respectively. Reduction of **2** also afforded **14**. Berberine [**1**] was reduced via dihydroberberine to **3** which was *N*-methylated to give rise to the α-*N*-metho salt [**4**]. Conversion of **4** via **6** into **7** has been confirmed in *C. ophiocarpa* (8).

13-Methylpalmatine [**9**], tetrahydropalmatine [**10**], and corydaline [**20**] were identified in Experiment 3 in which palmatine [**8**] was fed (Figure 3). Deuterated muramine (**13**-d_3), **13**, and tetrahydropalmatine α-*N*-metho salt [**11**] as well as **8** and **9** were recognized in Experiment 4 in which tetrahydropalmatine [**10**] was administered (Figure 3). The metabolic sequence **20**, **9**←**8**↔**10**→**11**,**13** was thus demonstrated in the callus of *C. pallida* var. *tenuis*. Palmatine [**8**] was bioconverted to **9** and **20** and also reduced to **10** which was biotransformed to the α-*N*-metho salt [**11**] in a manner similar to **1**. Conversion of **11** into **13** parallels that of **4** into **6**.

Thalictricavine [**14**] and its α-*N*-metho salt [**15**] were detected in Experiment 5, in which 13-methylberberine [**2**] was fed (Figure 4). 13-Methylberberine [**2**] and mesothalictricavine [**18**] or [**14**] were identified in Experiments 6 and 7, in which thalictricavine [**14**] and mesothalictricavine [**18**], respectively, were fed (Figure 4). The metabolic transformations **18**→**2**↔**14**, **15** were thus validated in the callus of *C. pallida* var. *tenuis*. Both *cis*- and *trans*-13-methyltetrahydroprotoberberines, thalictricavine [**14**], and mesothalictricavine [**18**], were oxidized to furnish 13-methylberberine [**2**]. 13-Methylberberine [**2**] was reduced to **14** which underwent *N*-methylation to produce the α-*N*-metho salt [**15**].

Corydaline [**20**] was identified in Experiment 8 in which 13-methylpalmatine [**9**] was administered (Table 2). 13-Methylpalmatine [**9**] and mesocorydaline [**23**] or [**20**] were detected in Experiments 9 and 10 in which **20** or **23**, respectively, were fed (Table 2). The metabolic conversions **23**→**9**↔**20** were thus proved in the callus of *C. pallida* var. *tenuis*. Corydaline [**20**] and mesocorydaline [**23**] undergo oxidation to 13-methylpalmatine [**9**] which can be reduced to generate **20**.

The new metabolic transformations, viz., a [**1**→**2**, **8**→**9**], c [**18**→**2**, **23**→**9**], d [**2**→**14**, **9**→**20**], e [**14**→**2**, **20**→**9**], g [**1**→**3**, **8**→**10**], and i [**10**→**11**] (Scheme 2) were all demonstrated by the results of Experiments 1–10. The known metabolic conversions (4,5), h [**3**→**1**, **10**→**8**] and i [**3**→**4**], were also confirmed in cultured cells of *C. pallida* var. *tenuis*. The metabolic transformations in *C. pallida* var. *tenuis* demonstrated in the present investigations are summarized in Scheme 2.

Feeding experiments using unlabeled protoberberines (Table 3, Experiments 11–20) were carried out in cultured cells of *C. incisa* as described for those on *C. pallida* var. *tenuis*. Each extract fraction (Chart 2) was subjected to lc-apcims (SIM method). The

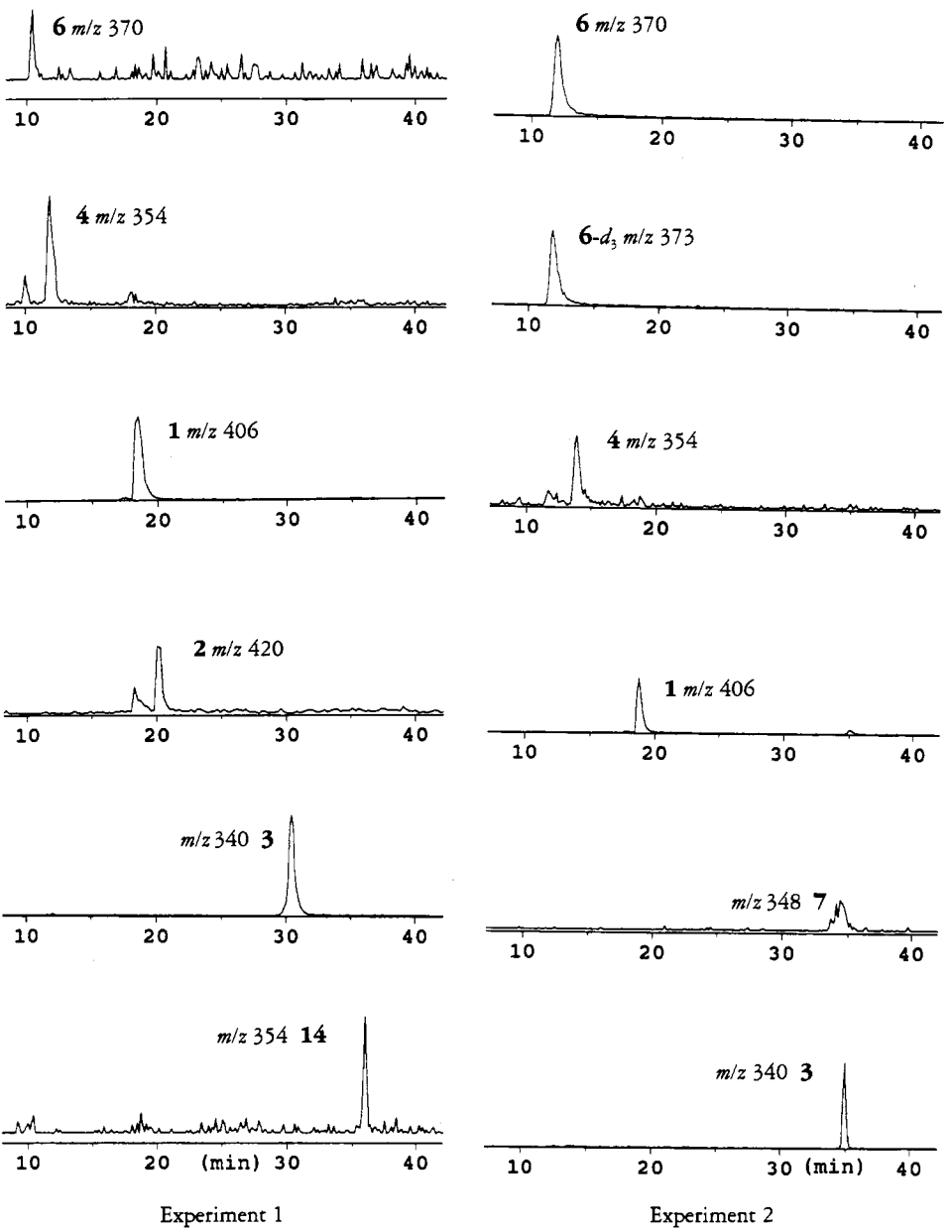


FIGURE 2. Identification of the metabolites obtained from Experiments 1 [feeding of berberine [**1**]] and 2 [feeding of tetrahydroberberine [**3**]] by ions [*m/z*] observed by lc-apcims. The molecular ions of the expected metabolites were monitored by mass chromatography [SIM method, nebulizer temperature: 320°]. The differences in retention times between Experiments 1 and 2 may be due to the measurement conditions of lc [concentration of TFA, etc.].

metabolites identified in each fraction that were obtained from Experiments 11–20 are summarized in Table 3.

Allocryptopine [**6**] was identified in Experiment 11 in which berberine [**1**] was fed (Table 3). Berberine [**1**], tetrahydroberberine α-*N*-metho salt [**4**], deuterated allocryptopine [**6**-d_3], and **6** were identified in Experiment 12 in which tetrahydroberberine [**3**] was administered (Table 3). The metabolic conversions **1**←**3**→**4**, **6** were thus

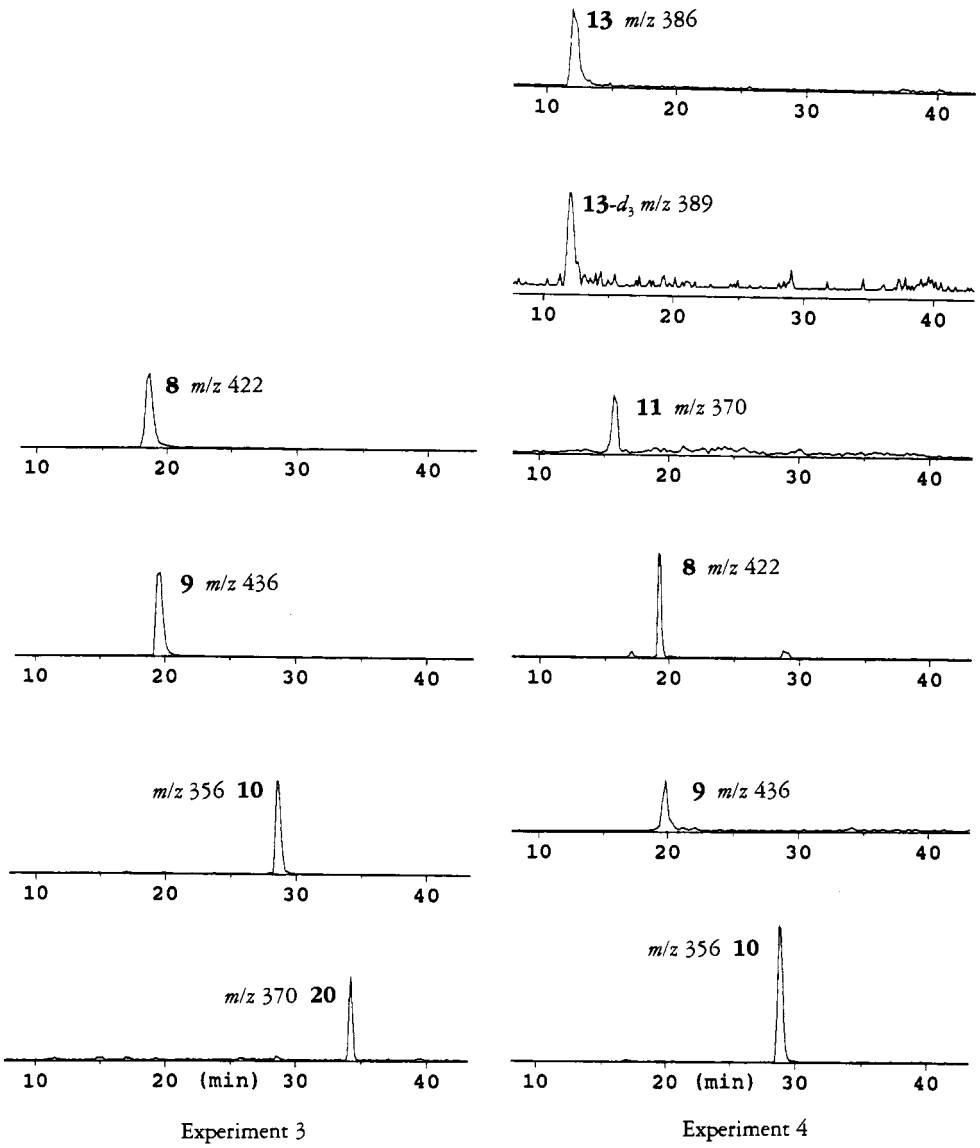


FIGURE 3. Identification of the metabolites obtained from Experiments 3 [feeding of palmatine [**8**]] and 4 [feeding of tetrahydropalmatine [**10**]] by ions [*m/z*] observed by lc-apcims. The molecular ions of the expected metabolites were monitored by mass chromatography [SIM method, nebulizer temperature: 300–340°].

demonstrated in cultured cells of *Corydalis incisa* (Pers.). The conversion of **4** into **6** has been previously demonstrated (8).

13-Methylpalmatine [**9**] was detected in Experiment **13**, in which palmatine [**8**] was fed. Palmatine [**8**], deuterated muramine [**13**-d_3], and **13** were characterized in Experiment 14 in which tetrahydropalmatine [**10**] was administered (Table 3). The metabolic sequence **9**←**8**→**10**, **13** was thus substantiated in the callus of *C. incisa*.

Thalictricavine [**14**], 13-methylallocryptopine [**17**], and mesothalictricavine [**18**] were detected in Experiment 15 in which 13-methylberberine [**2**] was fed (Table 3). 13-Methylberberine [**2**], thalictricavine α-*N*-metho salt [**15**], deuterated 13-methylallocryptopine [**17**-d_3], and **17** were identified in Experiment 16 in which

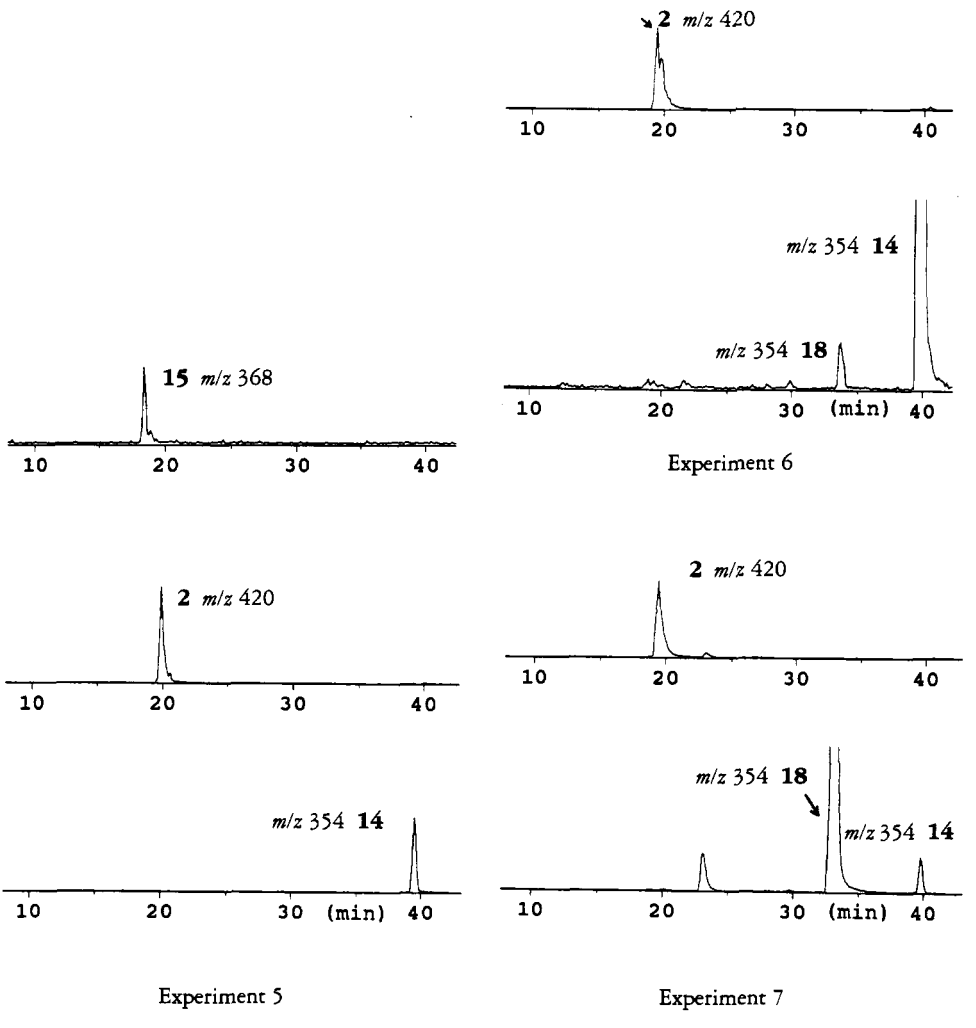


FIGURE 4. Identification of the metabolites obtained from Experiments 5 [feeding of 13-methylberberine [**2**], 6 [feeding of thalictricavine [**14**]] and 7 [feeding of mesothalictricavine [**8**]] by ions [*m/z*] observed by lc-apcims. The molecular ions of the expected metabolites were monitored by mass chromatography [SIM method, nebulizer temperature: 320°].

thalictricavine [**14**] was administered (Table 3). Mesothalictricavine α-*N*-metho salt [**19**] was found as well as **2**, **14**, **17**, and **17**-d_3 in Experiment 17, in which mesothalictricavine [**18**] was fed (Table 3). The metabolic conversions **17**, **19**←**18**↔**2**↔**14**→**15**, **17** were therefore demonstrated in the callus of *C. incisa*. 13-Methylberberine [**2**] underwent reduction to afford thalictricavine [**14**] and mesothalictricavine [**18**], which can be *N*-methylated to give rise to their α-*N*-metho salts [**15** and **19**], respectively, or can alternatively be oxidized to regenerate **2**. The transformation of **15** or **19** into 13-methylallocryptopine [**17**] has been previously demonstrated by Iwasa and co-workers (1).

Corydaline [**20**] was detected in Experiment 18 in which 13-methylpalmatine [**9**] was administered (Table 3). 13-Methylpalmatine [**9**] and mesocorydaline [**23**] or [**20**] were identified in Experiments 19 and 20 in which **20** and **23**, respectively, were utilized (Table 3). Hence, the metabolic transformations **23**→**9**↔**20** were substantiated in the cells of *C. incisa*.

TABLE 3. Administration of Protoberberine Alkaloids to Cell Cultures of *Corydalis incisa*.

Experiment	Wt of dry cells (g)	Medium (ml)	Substrates (mg)		Incubation time (days)	Metabolite(s) detected [observed ions (*m/z*)]
11	1.37	800	**1**	100	21	**6** [370]
12	1.86	400	(±)-**3**	50	21	**1** [406], **4** [454], **6** [370], **6**-d_3 [373]
			L-[Me-d_3]-Met[a]	25		
13	1.23	800	**8**	100	21	**9** [436]
14	2.78	400	(±)-**10**	50	21	**8** [422], **13** [386], **13**-d_3 [389]
			L-[Me-d_3]-Met[a]	25		
15	1.24	400	**2**	50	28	**14** [354], **17** [384], **18** [354]
16	2.24	400	(±)-**14**	55	10	**2** [420], **15** [368], **17** [384], **17**-d_3 [387]
			L-[Me-d_3]-Met[a]	25		
17	2.08	400	(±)-**18**	55	10	**2** [420], **14** [354], **17** [384], **17**-d_3 [387], **19** [368]
			L-[Me-d_3]-Met[a]	25		
18	1.24	400	**9**	50	28	**20** [370]
19	3.30	400	(±)-**20**	55	10	**9** [436], **23** [370]
			L-[Me-d_3]-Met[a]	25		
20	3.48	400	(±)-**23**	55	10	**9** [436], **20** [370]
			L-[Me-d_3]-Met[a]	25		

[a] L-[Me-d_3]-Methionine.

The new metabolic conversions, viz., a [**8**→**9**], b [**2**→**18**], c [**18**→**2**, **23**→**9**], d [**2**→**14**, **9**→**20**], e [**14**→**2**, **20**→**9**], f [**14**→**15**], and l [**18**→**19**] (Scheme 2) have been demonstrated by the results of Experiments 11–20. The known metabolic conversions (4,5), h [**3**→**1**, **10**→**8**] and i [**3**→**4**] were also confirmed in cultured cells of *C. incisa*. The biotransformation pathway in *C. incisa* deduced from the present work is summarized in Scheme 2.

There are certain differences in metabolism between cultured cells of *C. pallida* var. *tenuis* and those of *C. incisa*. In the former, the bioconversion (route k in Scheme 2) of tetrahydroprotoberberines [**4**] devoid of a C-13 methyl group, via protopine bases [**6**] into benzophenanthridines [**7**] does take place, but that (route m in Scheme 2) of the 13-methyltetrahydroprotoberberines [**15**] to the 13-methylprotopines [**17**] was not detected. In the latter, the biotransformation of **15** into **17** and of the 13-methylated protopines corycavine [**26**] (Chart 1) into the benzophenanthridines corynoline [**27**] (Chart 1) (9) do occur, but the biotransformation of **6** to **7** was not observed. These findings show that the metabolism of protoberberines without a methyl group at C-13 and those with a methyl group at that site proceed preferentially in *C. pallida* var. *tenuis* and *C. incisa*, respectively. In other words, corycavine [**26**] and corynoline [**27**] with a *C*-methyl group were isolated from *C. incisa*, but not from *C. pallida* var. *tenuis* (2).

The metabolism of the quaternary protoberberine alkaloids in cultured cells of *Corydalis* spp. has been clarified by lc-apcims. Interconversions, via redox reactions, of tetrahydroprotoberberines [**3** and **10**] or *cis*- and *trans*-13-methyltetrahydroprotoberberines [**14**, **20** and **18**, **23**] and their dehydro derivatives, protoberberinium salts [**1** and **8**] or 13-methylprotoberberinium salts [**2** and **9**], were demonstrated. α-*N*-Metho salts [**4**, **11**, **15** and **19**] incorporating the B/C-*cis* quinolizidine system were produced from tetrahydroprotoberberines or *cis*- or *trans*-13-methyltetrahydroprotoberberines [**3**, **10**, **14**, and **18**]. Finally, *C*-methylation at C-13 of protoberberinium salts [**1** and **8**] was confirmed to generate 13-methylprotoberberinium salts [**2** and **9**].

EXPERIMENTAL

GENERAL EXPERIMENTAL PROCEDURES.—^{1}H-nmr spectra were obtained on a Varian VXR-500S 500 MHz spectrometer using CD_3OD as solvent. Mass spectra were determined on a Hitachi M 80 instrument at 75 eV.

LC/APCI-MS METHOD.—Lc-apcims was carried out using a Hitachi M-1000H connected to a Hitachi L-6200 intelligent pump and a Hitachi L-4000 uv detector. Lc was performed on a Cosmosil 5 C_{18}-AR (4.6 mm i.d.×150 mm) reversed-phase column. The mobile phase was 0.1 M NH_4OAc (0.05% TFA, A), to which MeOH (B) was added by a linear gradient: initial (0% of B) −10 min, 30% of B, 15 min, 50% of B, 20–25 min, 60% of B, 30 min, 70% of B, 35 min, 80% of B, 40 min, 30% of B. The flow rate was 1 ml/min. Uv 280 nm. Apcims conditions: nebulizer and vaporizer temperatures were 300°–340° and 399°, respectively. Drift voltage was 20 V. The quasimolecular ions were monitored with the SIM method.

PLANT MATERIAL.—The calli of *C. pallida* var. *tenuis* and *C. incisa* were derived from the stems of wild grown plants in Kobe, Japan on Murashige and Skoog's medium (10) containing 2,4-dichlorophenoxyacetic acid (1 mg/l), kinetin (0.1 mg/l), yeast extract (0.1%), and agar (1%), in 1988 and 1989, respectively. The callus tissues were subcultured every 3 or 4 weeks on the same fresh medium at 25° in the dark. L-[Me-d_3]Methionine (99%) was purchased from Aldrich. Tetrahydroprotoberberines **3** and **10** were prepared by reduction of protoberberinium salts **1** and **8**, respectively. *cis*- and *trans*-13-Methyltetrahydroprotoberberines (**14**, **18**, **20**, and **23**) were prepared according to the method of Takao *et al.* (3). The α- and β-*N*-metho salts of protoberberines (**4**, **5**, **11**, **12**, **15**, **16**, **19**, **21**, **22**, **24**) were obtained according to the procedure described in previous papers (1,11). Allocryptopine [**6**] and chelerythrine [**7**] were natural products from *Macleya cordata*. Muramine [**13**] was obtained from *Papaver nudicaule*. The 13-methyl-[*N*-$^{13}CH_3$] protopines **17** and **25** are metabolites obtained from feeding experiments of the α-*N*-metho salts of [*N*-$^{13}CH_3$]-thalictricavine and [*N*-$^{13}CH_3$]-corydaline, respectively, to *C. incisa* (1).

PREPARATION OF 13-METHYLBERBERINE CHLORIDE [**2**].—A mixture of acetoneberberine (7.68 g) (12) in Me_2CO (200 ml) and MeI (10 ml) was placed in a glass-stoppered bottle and heated for 4 h at 85°. After

the mixture had cooled, the resulting crystals were filtered and treated with AgCl in MeOH to convert them into the chlorides (4.68 g), which are a mixture of berberine [**1**] and 13-methylberberine [**2**]. The mixture of chlorides (1 g) was heated in MeOH and the crystals (420 mg) in soluble in hot MeOH were filtered out. After cooling, the chlorides (500 mg) obtained from this filtrate were recrystallized from MeOH to give **2** (280 mg): mp 192–198° (dec); ^{1}H nmr δ 2.99 (3H, s, Me-13), 3.16 (2H, t, J=5.5 Hz, H_2-5), 4.12 and 4.21 (3H each, s, OMe×2), 4.81 (2H, t, J=5.5 Hz, H_2-6), 6.12 (2H, s, OCH_2O), 7.03 (1H, s, H-4), 7.34 (1H, s, H-1), 8.15 (2H, s, H-11 and H-12), 9.78 (1H, s, H-8); eims *m/z* $[M-HCl]^+$ 349 (19), 335 (100), 320 (30); hrms *m/z* $[M-HCl]^+$ 349.1316 ($C_{21}H_{19}O_4N$ requires 349.1313), 335.1162 ($C_{20}H_{17}O_4N$ requires 335.1157).

PREPARATION OF 13-METHYLBERBERINE CHLORIDE [**9**].—A mixture of acetonepalmatine (9.25 g) (12) in Me_2CO (200 ml) and MeI (10 ml) was treated as described in the preparation of **2** to afford the chlorides (6.96 g). The chlorides (700 mg) were recrystallized from MeOH to give **9** (420 mg): mp 186–195° (dec); ^{1}H nmr δ 3.04 (3H, s, Me-13), 3.19 (2H, t, J=5.5 Hz, H_2-5), 3.93, 3.97, 4.13, and 4.22 (3H each, s, OMe×4), 4.83 (2H, t, J=5.5 Hz, H_2-6), 7.14 (1H, s, H-4), 7.40 (1H, s, H-1), 8.15 and 8.16 (1H each, d, J=8.5 Hz, H-11 and H-12), 9.79 (1H, s, H-8); eims *m/z* $[M-HCl]^+$ 365 (2), 351 (100), 336 (31); hrms *m/z* $[M-HCl]^+$ 365.1622 ($C_{22}H_{23}O_4N$ requires 365.1625), 351.1476 ($C_{21}H_{21}O_4N$ requires 351.1470).

FEEDING EXPERIMENTS.—Feeding experiments (Tables 2 and 3) were carried out as described below. Substrates were dissolved in H_2O (2–4 ml) and introduced into 100-ml conical flasks containing 40 ml of autoclaved MS medium, which is the same as that employed in the subculture, through a steric bacterial filter. Calli (ca. 4–5 g) were transferred to each conical flask and incubated at 25° in the dark for an appropriate amount of time (Table 2). Cells and/or medium were separated and extracted with H_2O/MeOH at 50°. Extracts were worked up as described in Chart 2.

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